ELEKTRONENMIKROSKOPISCHES ZENTRALLABORATORIUM



DER UNIVERSITÄT ZÜRICH

Gloriastrasse30 Postfach CH-8028 Zürich Telefon 01 - 257 26 01 Fax 252 81 07 Bitnet K226110@CZHRZU1A

Dr. Markus Dürrenberger

Dr.Joshua Lederberg ROCKEFELLER UNIVERSITY 1230 York Avenue NEW YORK, NY 10021-6399

USA

Tel. (01) 257 26 03

Zürich, 3.7.90

Dear Prof. Lederberg,

Thank you for your note and the research profile. I do not see any problems to give you any pictures you like for publication, since you are a kind of the father of conjugation. You will receive a selection of micrographs and LM pictures. I appologise that it has become much easier with modern instruments to get nice pictures than it was at the time you discovered conjugation. Lately I followed several Hfr-conjugations with video enhanced light microscope and made a live video. The donors were immobilized to the cover slip and the recipients were added by flow through with the medium. It was a kind of fascinationg to see a full cycle live (You will get videoprints from that film). 3D EM-computer reconstruction is half way through, but not finished (more work than light microscopy).

To your questions: There is, as far as I know, no reasonable way to visualize DNA on thin sections. The best expected EM-resolution is about 5nm laterally. As markers one could use radioactively labeled DNA and prepare the thin sections with an emulsion that is developed similar to X-ray film treatment, but the resulting silver grains are up to 50nm which does not give a clear proove of transfer.

The conclusion I have drawn from the junction is the following: Several proteins are coded on the transferable plasmid that are located in the inner membrane, the periplasmic gel and the outer membrane of the donor. Their function is certainly connected to DNA transfer and they might be part of a conjugational junction. Therefore no qualitatively new substances are required. The recipient needs as factors that are known ompA and a functional LPS. If you think through the conjugational junction, you end up with

defined pores until the DNA has reached the inner membrane of the recipient. All these presumptive structures are below EM resolution of thin sections.

How does the DNA pass the inner membrane of the recipient and replicate to ds DNA? I remembered transformation. There are certain people suspecting a sugar uptake-system to be responsible for DNA uptake. So nothing easier than testing for this hypothesis. I made matings with the Glucose- and Manose uptake mutants of Rosenbusch / Erni as recipiens. There was about 50% inhibition. I decided not to take this as significant difference, because there were experimental incertainties by comparing sugar uptake deficiant strains that suffer of energy problems to control strains. I could not test double mutants (not existing) or other uptake deficient mutants. So I keep this idea in mind, but do not dare to give an answer.

At this place I would like to thank you for your interest in my findings. I hope that I can present my pictures at the meeting of Dr. Paranchych in Banff in September. I still do not have the permission of the University of Zürich (my position is too low to travel around on my own).

With best regards

W. Harrerly